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# A randomized, double-blind, placebo controlled, parallel group, efficacy study of alpha BRAIN® administered orally

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**Objective** Alpha BRAIN® is a nootropic supplement that purports to enhance cognitive functioning in healthy adults. The goal of this study was to investigate the efficacy of this self-described cognitive enhancing nootropic on cognitive functioning in a group of healthy adults by utilizing a randomized, double blind, placebo-controlled design.
**Methods** A total of 63-treatment naïve individuals between 18 and 35 years of age completed the randomized, double-blind, placebo controlled trial. All participants completed a 2-week placebo run in before receiving active product, Alpha BRAIN® or new placebo, for 6 weeks. Participants undertook a battery of neuropsychological tests at randomization and at study completion. Primary outcome measures included a battery of neuropsychological tests and measures of sleep.
**Results** Compared with placebo, Alpha BRAIN® significantly improved on tasks of delayed verbal recall and executive functioning. Results also indicated significant time-by-group interaction in delayed verbal recall for the Alpha BRAIN® group.
**Conclusions** The use of Alpha BRAIN® for 6 weeks significantly improved recent verbal memory when compared with controls, in a group of healthy adults. While the outcome of the study is encouraging, this is the first randomized controlled trial of Alpha BRAIN®, and the results merit further study. Copyright © 2016 John Wiley & Sons, Ltd.

KEY WORDS—randomized controlled trial; nootropic; nutraceutical; cognitive enhancement

## INTRODUCTION

In 2012, supplement users in the United States exceeded 40 million people and generated approximately 30 billion dollars in revenue (Ooyen, 2012; Wu *et al*., 2014). In concert with these figures, supplements purporting to provide cognitive benefits, known as 'nootropics' (Giurgea, 1972; Chapman *et al*., 2012; Wang, 2012), also have experienced increased popularity and sales despite research demonstrating mixed or limited benefit from their use (Solomon *et al*., 2002; Hirsch, 2012).

Much of the recent growth in popularity of nootropics and other cognitive enhancers has been documented among young, 18- to 35-year-old, cognitively healthy adults (McCabe *et al*., 2005; DeSantis *et al*., 2008; Maher, 2008; Tablot, 2009; Smith and Farah, 2011), and although the consumption and regulation of supplements, including nootropics, remains a controversial topic (Shook *et al*., 2014), there remains a considerable interest in the potential role of supplements, nootropics and other therapeutics in aiding the cognitive performance of adults (Zangara, 2003; Shineman *et al*., 2010; Dietz *et al*., 2013).

Elucidating the specific effects of nootropics in cognitively healthy populations is a primary step in determining whether these products provide clinical benefit. While drugs that are considered for approval by the United States Food and Drug Administration (FDA) must adhere to a strict protocol to document both safety and efficacy before dissemination (Leber, 1990), there is no current set of federal guidelines by which to judge the efficacy of nootropics (Solomon and Michalczuk, 2009), which has led to a varied and arguably insufficient amount of reliable and valid data to accurately characterize their efficacy (Solomon and Michalczuk, 2009).

Alpha BRAIN® (Onnit Labs LLC) is a multi-ingredient nutritional supplement that purports to enhance cognitive function in healthy adults. The

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commercially available product contains 12 naturally occurring compounds (Figure 1) including L-tyrosine; huperzine A, bacopa, phosphatidylserine and L-alpha glycerylphosphorylcholine; vinpocetine and pterostilbene. Each compound present in the Alpha BRAIN® formula has evidence to support safe consumption and provide possible cognitive benefit (Barbagallo Sangiorgi *et al*., 1994; Sun *et al*., 1999; Sheng *et al*., 2001; Valikovics, 2007; Berry *et al*., 2011; Downey *et al*., 2013; Unno *et al*., 2013; Hirayama *et al*., 2014; Song *et al*., 2015; Steenbergen *et al*., 2015; Yang *et al*., 2015). In addition, the manufacturer has suggested that their specific formulation of compounds may increases acetylcholinergic neurotransmission and provide neuroprotective benefit. However, prior studies have been specific to the individual compounds found in Alpha BRAIN® and have not focused on the compound or formulation as a whole.

The purpose of the present study was to evaluate the efficacy of the nootropic Alpha BRAIN® on the manufactures purported target population of healthy adults utilizing a randomized, double-blind, placebo controlled trial using standardized tests of learning, memory, attention, concentration, processing speed and executive functioning as well as measures of sleep.

## MATERIALS AND METHODS

### *Participants*

Participants for the current study were recruited from the community using a targeted strategy. Recruitment methods included the distribution of posted advertisements as well as web-based recruitment. Recruitment materials provided a brief description of the research as well as contact information for the research center. Upon encountering recruitment materials, participants were instructed to call the research center to be screened for study eligibility.

Potential participants were screened for eligibility via telephone by a trained sub-investigator. Inclusion criteria included: (i) being between the ages of 18–35 inclusive; (ii) community dwelling, fluent in English and able to understand and sign informed consent; (iii) Mini Mental State Examination (MMSE) score $> 26$; and (iv) body mass index (BMI) within two standard deviations of normal. Exclusion criteria included: (i) any visual or auditory disability which would interfere with cognitive testing; (ii) no previous diagnosis of stroke, ADD/ADHD, learning disability or cardiac condition; (iii) no current or past (5 years) diagnosis of any neurologic, psychiatric or life-threatening illness; (iv) not currently (past 60 days) taking antidepressants or other psychoactive medication; (v) not currently (past 60 days) taking any non-prescription nutraceuticals, medical foods or vitamins which purported to have cognitive enhancing properties; and (vi) not currently dependent on any drugs or alcohol based on the MINI International Neuropsychiatric Interview (MINI) (Lecrubier *et al*., 1997).

### *Interventional compound and randomization schedule*

The study was double-blinded. Participants were randomly assigned to receive Alpha BRAIN® (Onnit Labs LLC) or matched placebo control. The Alpha BRAIN formula utilized for the study was the same commercially available formula that is currently

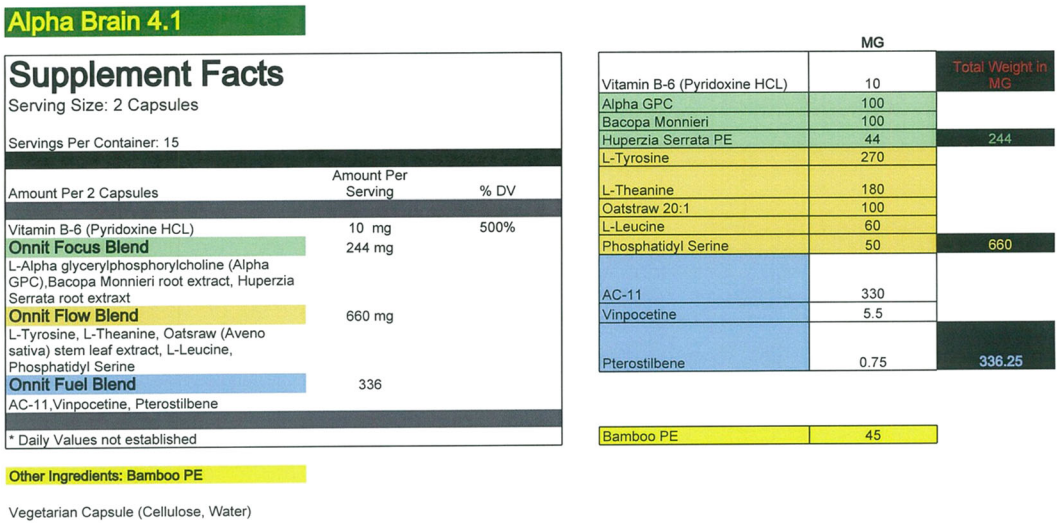


Figure 1. Alpha BRAIN® ingredients




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available to consumers. Participants followed the manufacturer's recommended dosing instructions of two capsules, preferably with food, per day. Random assignment of participants to each condition was done on a (1:1) ratio and determined by the primary investigator utilizing random number generation (Games and Klare, 1967).

### *Procedures*

An 8-week double-blind, placebo-controlled, parallel group, efficacy study was conducted at a single site. A total of 118 community dwelling participants were screened over a 10-month period from September 2013 to July 2014 of which 73 were randomized into the study. Figure 2 summarizes the study participation.

All procedures in the current study were approved by the New England Institutional Review Board (IRB), and all persons who participated in the in-person assessment completed informed consent. As described previously, initial contact with potential participants was done via a telephone screen where basic inclusion and exclusion criteria was assessed (e.g. age 18–35 years). If eligible, an in-person baseline visit was scheduled during which the participant went through the informed consent procedure followed by the MMSE. Participants were randomly assigned to 1 of 2 conditions: Alpha BRAIN® or placebo control. At baseline, all participants received a 14-day supply of placebo in a plain sealed and dated bottle as well as instructions regarding administration of the supplement. This placebo run-in was not disclosed to participants as it was used to help mitigate potential placebo effects and participants who might demonstrate poor adherence (Davis *et al*., 1995). Participants were scheduled to return approximately one day after finishing the placebo-run in, but were encouraged to make contact with research team if any adverse events (AEs) transpired prior to returning.

At day (+15), participants returned to the research center and completed the cognitive assessment battery, returned their pill bottles and had any unreported AEs documented. At the end of this visit, participants were given a new 6-week supply of either Alpha BRAIN® or placebo in a plain, sealed bottle and instructed to follow the same administration procedure. Once again participants were encouraged to contact the study team should any AE transpire. Approximately 6 weeks later, +/− 3 days, subjects returned to repeat the cognitive assessment battery. Pill bottles were once again collected, and any unreported AEs were documented.

### *Measures*

In addition to the measures of cognitive performance described below, participants completed several additional instruments at the screening assessment. These included a questionnaire on basic demographic information and a screen of global cognitive performance (MMSE) (Crum, 1993).

### *Cognitive measures*

The battery of cognitive tests included standard measures that were broadly categorized into several areas —learning and memory, attention, concentration and processing speed and executive functioning. Participants were also administered measures with regard to wakefulness and dreams.

Premorbid intellectual ability was determined using the Wechsler Test of Adult Reading (WTAR) (Wechsler, 2001). The WTAR asks participants to pronounce a series of 50 irregularly spelled words. This procedure continues through all 50-word cards and is discontinued if the patient provides 12 consecutive incorrect pronunciations. WTAR scores are shown to

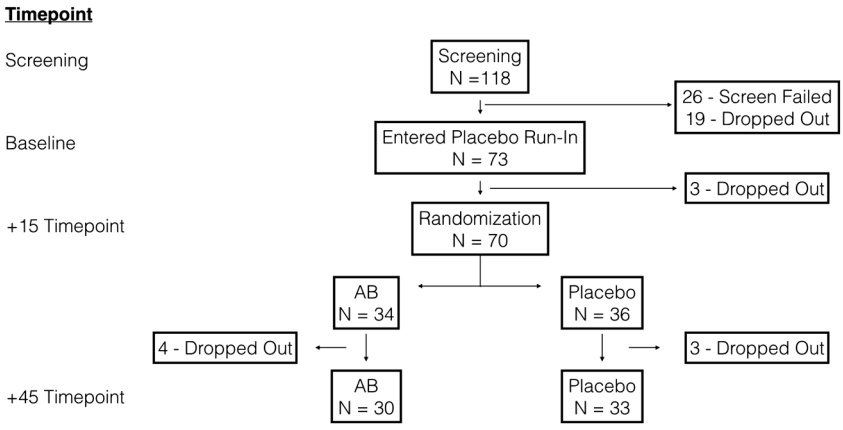


Figure 2. Disposition of the sample




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correlate highly with measures of both verbal and full scale IQ (Strauss *et al*., 2006).

Tests of learning and memory included the Logical Memory subscale of Wechsler Memory Scale IV (WMS-IV) (Wechsler, 2009) in which participants are asked to recall paragraphs both immediately and after a 30-min delay; the Symbol Span subtest of WMS-IV which asks participants to study and then recognize a sequence of increasingly complex shapes; a modified version of the California Verbal Learning Test II (CVLT-II) (Wodds *et al*., 2001) in which the participant is asked to learn a 16-item shopping list over several trials and then to subsequently recall that information after both a short delay with distractor and a long delay and the Brief Visual Memory Test —Revised (BVMT-R) (Benedict *et al*., 1996) in which participants are asked to study a set of designs over several trials and then accurately reproduce them both immediately and after a 25-min delay.

Tests of attention, concentration and processing speed included the Digit Symbol subscale of the Wechsler Adult Intelligence Scale IV (WAIS-IV) (Wechsler, 2008) in which the participant must rapidly copy symbols associated with numbers; The Stroop test (Jensen and Rohwer, 1966) in which requires participants to keep from being distracted by extraneous aspects of the stimuli; the Trials Making A Test (Reitan, 1958) which involves drawing a line connecting consecutive numbers from 1 to 25 as quickly as possible and the Paced Serial Addition Test (PASAT), (Tombaugh, 2006) which involves presenting the participant a series of single digit numbers read out loud where the two most recent digits must be summed prior to the presentation of the next digit.

Tests of executive functioning included the Trails Making B Test (Reitan, 1958) which asks participants to draw a line connecting alternating numbers and letters in sequence as quickly as possible; the Delis Kaplan Executive Function (DKEFs) Letter Fluency subtests(Delis *et al*., 2001) which asks participants to name as many words that begin with a certain letter that they can think of over a 1-min period; the DKEFs Category Fluency subtest (Delis *et al*., 2001) which similarly asks participants to name as many words that fit into a specific category (animals) over a 1-min period and the DKEFs 20-Questions subtest (Delis *et al*., 2001) in which participants attempt to guess an object the examiner has selected using yes/no questions and modify their guesses based on examiner feedback.

Last, participants filled out both the Epworth Sleepiness Scale (Johns, 1991) which instructs participants to rate their likelihood of falling asleep on a scale from: (0) no chance of dozing to (3) high chance of dozing and the Mentation Report (Nielsen, 2000) which asks participants to rate their emotional state when they awoke as well as note if they remembered experiencing any vivid imagery while sleeping.

### *Statistical analysis*

Considerations of sample size were based on statistical power analysis (Cohen, 2013). Specifically, based on prior data from an initial pilot study of Alpha BRAIN® (Solomon *et al*., 2014) this protocol was powered to detect a medium ($d = 0.05$) to large effect ($d = 0.8$) for an independent samples test with a total sample size of 30 subjects per group. A primary analysis of efficacy was performed on the on fully evaluable population, defined as individuals who completed the 15 days of placebo run-in and 45 days of double-blind treatment and who satisfactorily completed the standards for supplement adherence. Differences in-group means were assessed both by individual *t*-tests and by repeated measures analysis of variance (ANOVA) in which the treatment condition serves as the predictor and the cognitive test serves as the measure. The test by condition-interaction term was used to test for statistical significance. Demographic variables were tested by individual *t*-tests. Categorical variables were tested by $\chi^2$. All statistical analyses were considered significant if they reached the 0.05 level. SPSS Version 20 (Nie *et al*., 1975) statistical software was used for all analysis.

## RESULTS

A total of 73 participants were enrolled in the study over a 10-month period, with a total of 63 completing the study (Figure 2). The percentage of participants who completed the study did not differ significantly by treatment group. Of the 10 participants who did not complete the study three withdrew before beginning randomization, four were from the Alpha BRAIN® group and three from the placebo group. The majority of persons who left the study indicated they did not have time to participate and withdrew consent.

Demographic characteristics of the sample that completed the study are shown in Table 1. The sample was approximately 23 years of age and had slightly more women ($n = 35$) then men ($n = 28$). The majority of the sample was white, currently enrolled in college and had an MMSE over 29. Using both *t*-tests and chi-square tests to compare treatment groups on




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Table 1. Demographic characteristics of the study sample

| | Total $n=63$ | Alpha BRAIN $n=30$ | Placebo $n=33$ |
|---|---|---|---|
| *Age at baseline* | $M=23.67$ | $M=24.43$ | $M=22.97$ |
| *Estimated IQ (WTAR)* | $M=110.19$ | $M=110.73$ | $M=109.69$ |
| *MMSE score* | $M=29.48$ | $M=29.50$ | $M=29.45$ |
| *Gender* | % (*n*) | % (*n*) | % (*n*) |
| Male | 44.4 (28) | 46.7 (14) | 42.4 (14) |
| Female | 55.6 (35) | 53.3 (16) | 57.6 (19) |
| *Level of education* | | | |
| Less than high school | 3.2 (2) | 6.7 (2) | 0.0 (0) |
| High school | 19.0 (12) | 20.0 (6) | 18.2 (6) |
| Associates degree | 7.9 (5) | 13.3 (4) | 3.0 (1) |
| Current college student | 50.8 (32) | 40.0 (12) | 60.6 (20) |
| Bachelors degree | 15.9 (10) | 20.0 (6) | 12.1 (4) |
| Graduate degree | 3.2 (2) | 0.0 (0) | 6.1 (2) |
| *Race/Ethnicity* | | | |
| White (Not Hispanic) | 69.8 (44) | 63.3 (19) | 75.8 (25) |
| Black/African American | 4.8 (3) | 6.7 (2) | 3.0 (1) |
| Hispanic | 11.1 (7) | 6.7 (2) | 15.2 (5) |
| Asian/Pacific Islander | 7.9 (5) | 10.0 (3) | 6.1 (2) |
| American Indian | 1.6 (1) | 3.3 (1) | 0.0 (0) |
| Mixed/Multiple | 4.8 (3) | 10.0 (3) | 0.0 (0) |

MMSE, Mini Mental Status Exam; *M*, mean; WTAR, Wechsler Test of Adult Reading.

demographic variables revealed no statistically significant differences.

### *Cognitive performance*

A mixed model repeated measures ANOVA was performed for each cognitive outcome measure. Results of the ANOVA indicated a significant test-by-treatment interaction for CVLT Long Delay ($F$ [1,61]$=4.07$, $p=0.04$, partial eta squared$=0.06$.) with the Alpha BRAIN® group demonstrating significantly improved performance over placebo control (see Figure 3). No other group effects reached statistical significance.

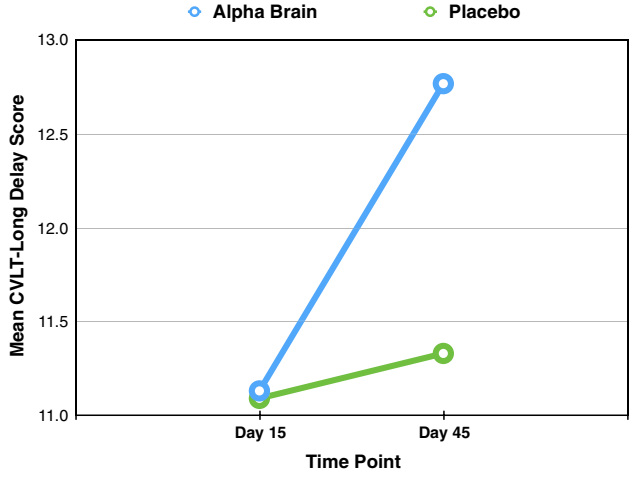


Figure 3. Test-by-treatment interaction for CVLT long delay

Table 2 displays the means and standard deviations for each of the cognitive outcome measures at the +15 and +45 day time-points. Between groups analysis of results indicated that after two weeks of placebo run-in, no significant differences were found between groups on any cognitive outcome measure or measure of sleep. Following randomization to the Alpha BRAIN® or placebo groups, results indicated a significant difference on two cognitive measures. Specifically, those participants randomized to the Alpha BRAIN® group demonstrated improved performance on Trail Making Test B ($t$ [61]$=-1.96$, $p=0.05$, partial eta squared$=-0.49$) and CVLT Long Delay Recall ($t$ [61]$=2.48$, $p=0.01$, partial eta squared$=0.63$).

Finally, within group analysis indicated that, in general, participants in both groups tended to perform better during their second evaluation than during their first. Participant's mean scores improved on a number of cognitive outcome measures including Logical Memory immediate and delayed recall, Brief Visual Memory Task, Trials Making Test A and B, CVLT, Stroop and PSAT. Superior performance by both groups at the second testing session was likely a result of a practice effect (Solomon *et al*., 2002).

### *Compliance and adverse effects*

Compliance with supplement adherence was documented following the placebo run-in as well as the 6 weeks of randomized treatment. At both time-points, adherence to study supplement was excellent (>90%) with no significant differences in adherence rates as a function of group, post placebo run in, ($t$ [61] $=-0.85$, $p=0.40$), end of study, ($t$ [61]$=-3.71$, $p=0.07$).

Similarly, all AEs were also documented. Overall, less than 2% of participants in the study noted any AEs during the placebo run-in period. Roughly 9% of participants indicated an AE during the randomized treatment period. The most common complaints included headache, upset stomach and vivid dreams. No serious AEs were reported by any participant. Analysis indicated that no significant differences were found between study groups with regard to amount of AEs reported at either time point, post placebo run in ($\chi^2$ (1)$=0.924$ $p=0.336$), end of study, ($\chi^2$ (1) $=0.965$ $p=0.326$).

## DISCUSSION

Results of the 8-week study indicate modest improvement to performance in verbal memory (CVLT-Long Delay) for those participants taking Alpha BRAIN®, as compared to those randomized to placebo, who

Table 2. Cognitive test score (mean and SD) from +15 and +45 time points

| | +15 | | +45 | |
|---|---|---|---|---|
| Outcome measure | AB $n = 30$ | Placebo $n = 33$ | AB $n = 30$ | Placebo $n = 33$ |
| Logical Memory I Total | $M = 27.27$ SD = 6.13 | $M = 26.00$ SD = 6.54 | $M = 29.57$ SD = 7.24 | $M = 28.57$ SD = 3.20 |
| Logical Memory II Total | $M = 23.67$ SD = 6.55 | $M = 22.88$ SD = 6.21 | $M = 28.57$ SD = 9.99 | $M = 29.45$ SD = 6.87 |
| BVMT Trial I | $M = 8.63$ SD = 2.78 | $M = 8.55$ SD = 2.27 | $M = 9.20$ SD = 3.03 | $M = 10.24$ SD = 2.20 |
| BVMT Trial II | $M = 10.90$ SD = 1.51 | $M = 11.09$ SD = 1.28 | $M = 10.93$ SD = 2.01 | $M = 11.42$ SD = 1.75 |
| BVMT Trial III | $M = 11.40$ SD = 1.07 | $M = 11.33$ SD = 1.36 | $M = 11.50$ SD = 1.13 | $M = 11.70$ SD = 1.05 |
| BVMT Total Trials I–III | $M = 31.10$ SD = 4.83 | $M = 31.00$ SD = 4.21 | $M = 31.37$ SD = 5.68 | $M = 33.36$ SD = 4.68 |
| BVMT Delay Trial | $M = 11.33$ SD = 1.12 | $M = 11.30$ SD = 1.21 | $M = 11.37$ SD = 1.21 | $M = 11.58$ SD = 1.03 |
| F Total | $M = 14.10$ SD = 4.11 | $M = 14.03$ SD = 4.01 | $M = 17.77$ SD = 5.63 | $M = 17.48$ SD = 4.57 |
| A Total | $M = 13.53$ SD = 3.90 | $M = 12.91$ SD = 3.61 | $M = 14.00$ SD = 4.88 | $M = 14.82$ SD = 4.83 |
| S Total | $M = 16.13$ SD = 3.73 | $M = 16.27$ SD = 4.30 | $M = 14.63$ SD = 4.18 | $M = 14.55$ SD = 4.43 |
| FAS Total | $M = 43.77$ SD = 5.30 | $M = 42.15$ SD = 10.1 | $M = 46.40$ SD = 12.9 | $M = 46.82$ SD 11.21 |
| Animals Total | $M = 24.63$ SD = 5.30 | $M = 24.55$ SD = 5.46 | $M = 22.27$ SD = 5.93 | $M = 22.58$ SD = 4.22 |
| Symbol Span | $M = 30.23$ SD = 7.07 | $M = 29.85$ SD = 9.14 | $M = 31.97$ SD = 9.11 | $M = 30.30$ SD = 6.88 |
| Digit Symbol Coding | $M = 58.50$ SD = 12.55 | $M = 59.64$ SD = 9.93 | $M = 58.23$ SD = 8.55 | $M = 64.12$ SD = 1.49 |
| Trails A | $M = 22.87$ SD = 8.78 | $M = 22.39$ SD = 5.69 | $M = 20.70$ SD = 6.57 | $M = 20.58$ SD = 6.80 |
| Trails B | $M = 54.90$ SD = 17.5 | $M = 62.55$ SD = 21.6 | $*M = 43.33$ SD = 15.1 | $*M = 51.21$ SD = 16.4 |
| 20 Questions Total Score | $M = 25.40$ SD = 5.21 | $M = 25.82$ SD = 5.37 | $M = 26.30$ SD = 4.42 | $M = 25.00$ SD = 5.33 |
| 20 Questions Ach. Score | $M = 16.60$ SD = 3.43 | $M = 16.33$ SD = 2.45 | $M = 15.73$ SD = 2.16 | $M = 16.03$ SD = 2.50 |
| Stroop Inhibition Trial | $M = 44.23$ SD = 9.00 | $M = 44.64$ SD = 10.7 | $M = 42.63$ SD 7.97 | $M = 40.55$ SD = 8.26 |
| Stroop Switching Trial | $M = 54.17$ SD = 13.6 | $M = 52.58$ SD = 9.61 | $M = 51.17$ SD = 13.8 | $M = 48.79 = 12.97$ |
| CVLT Total Score | $M = 43.76$ SD = 9.97 | $M = 43.21$ SD = 10.1 | $M = 46.40$ SD = 12.9 | $M = 46.84$ SD = 11.2 |
| CVLT Short Delay | $M = 11.27$ SD = 2.37 | $M = 11.39$ SD = 2.17 | $M = 11.83$ SD = 2.94 | $M = 12.39$ SD = 1.96 |
| CVLT Long Delay | $M = 11.13$ SD = 2.60 | $M = 11.09$ SD = 3.18 | $*M = 12.77$ SD = 2.2 | $*M = 11.33$ SD = 2.3 |
| PSAT Trial I | $M = 38.33$ SD = 13.18 | $M = 39.15$ SD = 11.8 | $M = 43.30$ SD = 13.0 | $M = 43.33$ SD = 10.3 |
| PSAT Trial II | $M = 28.83$ SD = 10.78 | $M = 28.64$ SD = 9.04 | $M = 30.03$ SD = 13.1 | $M = 32.12$ SD = 9.23 |
| Epworth Scale | $M = 10.50$ SD = 16.97 | $M = 28.64$ SD = 9.04 | $M = 7.23$ SD = 4.37 | $M = 7.72$ SD = 2.99 |

*<0.05
*M*: mean
SD: standard deviation
BVMT: Brief Visual Memory Test
CVLT: California Verbal Learning Test
PSAT: Paced Serial Addition Test

showed no such improvement. None of the other cognitive domains assessed demonstrated significant time-by group interactions over the study period. The results of the current randomized trial suggest that aspects of cognitive performance can be improved by this pill-based nootropic.

While small, the significant improvement in verbal memory demonstrated in this study are consistent with prior research that has examined the cognitive benefits of several individual nootropics found in Alpha BRAIN® (Huperzine A, *Bacopa monnieri* (BM)) as well as FDA approved pharmaceutical compounds (donepezil, galantamine, rivastigmine) on healthy adults. These individual compounds all share a similar mechanism of action that has been shown to improve memory through facilitating cholinergic synaptic transmission (Davis *et al*., 1979; Blokland, 1995; Beglinger *et al*., 2004; Morasch *et al*., 2015).

For example, prior studies have demonstrated the pharmacological profile of Huperzine A (HupA) as an effective acetylcholine esterase inhibitor (Skolnick, 1997; Morasch *et al*., 2015), and clinical studies in both healthy non-patient populations as well as individuals with diagnosed cognitive disorders (Xu *et al*., 1995; Xu *et al*., 1999; Zhang *et al*., 2002) have reported significant improvement in cognitive functioning, often specific to the area of memory.

In an often-cited double blind, matched-pair study, young adult students were randomized to receive either HupA or placebo for a duration of four weeks. Participants were evaluated using the Wechsler Memory Scale in addition to several other cognitive outcome measures. Results of the trial indicated that those participants receiving HupA demonstrated a significantly improved learning and memory performance and a sub analysis of WMS factors revealed that the HupA group experienced increased performance in scores of "accumulation," "recognition," "association," "factual memory" and "number of recitations" (Sun *et al*., 1999).

Empirical studies have also shown BM to have beneficial effects to cognition across multiple age groups (Pace, Kean, Sarris, Neale, *et al.*, 2012). In a 2008 randomized controlled trial, 54 cognitively healthy adults




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were randomized to receive either BM or placebo for 12 weeks following a 6-week placebo run in. Results of the study indicated that those participants randomized to the BM group demonstrated significant improvement in delayed verbal recall, compared to placebo, as evidenced by improved scores on the Rey Auditory Verbal Learning Test (AVLT) (Calabrese *et al*., 2008).

Likewise, a number of studies have reported cognitive benefit in young healthy adults when given the acetylcholine esterase inhibitor donepezil (Chuah and Chee, 2008; Chuah *et al*., 2009; Dodds *et al*., 2011). In a randomized, double-blind, placebo controlled, parallel group study, 30 young healthy male subjects who were randomized to receive donepizil or placebo for one month. Comprehensive neuropsychological testing was performed at baseline and again after the treatment period. Time-by-group interactions indicated that the group randomized to receive donepezil demonstrated significant improvement in both verbal and visual episodic memory (Grön *et al*., 2005).

The results described here demonstrated a comparably modest improvement in one domain of cognitive performance among a high-functioning group of healthy adults. The analysis of AEs and supplement compliance also indicated that the supplement was well tolerated by participants.

Although the results of the current study are informative, there are limitations to note. The participants examined here were very high functioning in terms of their overall cognitive abilities as well as general health and educational attainment. Given this, generalizing these findings to a larger population should be done with caution.

Notably, our study sample was inclusive to individuals aged 18–35. Given that individuals over 65 are the fastest growing portion of the US population (Budson and Solomon, 2011) and that a decline in cognitive functioning is considered to be a normal consequence of aging (Yurko-Mauro *et al*., 2010), future studies might find increased effects by studying older adults or adults with prior subjective cognitive complaints (Chapman *et al*., 2012; Small *et al*., 2014).

We recognize the possibility that ceiling effects also may have contributed to the non-significant findings; however, we selected tests that are normalized for the age group that was studied, and such, have an appropriate range of scores (Spreen, 1998). Further, despite our findings, our analysis was likely underpowered given the number of measures that were examined and future studies would benefit from larger overall sample.

Our 6-week intervention period may have been too short to observe more widespread cognitive benefit. We also did not include a follow-up assessment to observe if any differences in groups continued after study supplementation had ended. While we followed the manufacturer's suggestion for dosing, future studies should lengthen the intervention period and include a follow-up assessment to better evaluate optimal time and duration of results.

While a sub-sample of participants were included in an ERP/EEG study investigating biomarker changes associated with acute and long-term Alpha BRAIN® use (Cecchi *et al*., *under review*) future studies would benefit from following recommendations to evaluate subjective changes to cognitive functioning or activities of daily living as reported by a study informant (Solomon and Michalczuk, 2009).

Last, we are unable to draw conclusions regarding the mechanism of action for the effect we observed. While Alpha BRAIN® does include a number of compounds with reported cognitive benefit, the varying compounds, pharmacology, dosing, study designs and outcome measures utilized across prior studies of individual compounds make it difficult to generalize their results to those reported here (Scholey *et al*., 2005).

In light of the increasing availability and use of nootropic supplements in the United States and in concert with a lack of regulation and established standards for demonstrating efficacy, it is important to promote studies, such as this one, that utilize randomized, placebo-controlled trials and follow suggested evaluative guidelines for the study of cognitive enhancing supplements (Solomon and Michalczuk, 2009). While the present study demonstrated that use of the nootropic supplement Alpha BRAIN® for 6 weeks is well tolerated and resulted in a significant improvement to recent verbal memory in a group of healthy adults, this is the first randomized controlled trial of Alpha BRAIN®, and results will need to be replicated before any definitive conclusions regarding efficacy can be made.

## CONFLICT OF INTEREST

Solomon has received funds for consulting with Onnit; Leech has received funds for consulting with Onnit.

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## REFERENCES

Barbagallo Sangiorgi G, Barbagallo M, Giordano M, *et al.* 1994. alpha-Glycerophosphocholine in the mental recovery of cerebral ischemic

attacks. An Italian multicenter clinical trial. *Ann N Y Acad Sci* **717**: 253–269.
Beglinger LJ, Gaydos BL, Kareken DA, *et al.* 2004. Neuropsychological test performance in healthy volunteers before and after donepezil administration. *J Psychopharmacol* **18**(1): 102–108. DOI:10.1177/0269881104040248.
Benedict R, Schretlen D, Groninger L, *et al.* 1996. Revision of the Brief Visuospatial Memory Test: studies of normal performance, reliability, and validity. *Psychol Assess* **8**(2): 145–153.
Berry N, Robinson M, Bryan J, Buckley J, Murphy K, Howe P. 2011. Acute effects of an *Avena sativa* herb extract on responses to the Stroop Color–Word Test. *J Alternative Compl Med* **17**(7): 635–637.
Blokland A. 1995. Acetylcholine: a neurotransmitter for learning and memory? *Brain Res Rev* **21**(3): 285–300.
Budson A, Solomon P. 2011. *Memory Loss: A Practical Guide for Clinicians*. Elsevier Health Sciences: London.
Calabrese C, Gregory WL, Leo M, *et al.* 2008. Effects of a standardized *Bacopa monnieri* extract on cognitive performance, anxiety, and depression in the elderly: a randomized, double-blind, placebo-controlled trial. *J Alternative Compl Med* **14**(6): 707–713. DOI:10.1089/acm.2008.0018.
Cecchi M, Solomon TM, Leech JR, *et al*. in preparation. Evaluation of a handheld event related potential (ERP)/quantitative electroencephalography (qEEG) system (COGNISION™) as a useful cognitive biomarker for alpha BRAIN® administered orally.
Chapman SB, Cotman CW, Fillit HM, *et al.* 2012. Clinical trials: new opportunities. *J Gerontol A Biol Sci Med Sci* **67**(7): 773–780. DOI:10.1093/gerona/gls126.
Chuah L, Chong DL, Chen AK, Rekshan WR, III. 2009. Donepezil improves episodic memory in young individuals vulnerable to the effects of sleep deprivation. *Sleep* **32**(8): 999.
Chuah LYM, Chee MWL. 2008. Cholinergic augmentation modulates visual task performance in sleep-deprived young adults. *J Neurosci* **28**(44): 11369–11377. DOI:10.1523/JNEUROSCI.4045-08.2008.
Cohen J. 2013. *Statistical Power Analysis for the Behavioral Sciences*. Academic Press: New York, NY.
Crum R. 1993. Population-based norms for the Mini-Mental State Examination by age and educational level. *J Am Med Assoc* **269**(18): 2386–2391.
Davis C, Applegate W, Gordon D, Curtis R, McCormick M. 1995. An empirical evaluation of the placebo run-in. *Control Clin Trials* **16**(1): 41–50.
Davis K, Mohs R, Tinklenberg J, Pfefferbaum A, Hollister L, Kopell B. 1979. Physostigmine: improvement of long-term memory processes in normal humans. *Surv Anesthesiol* **23**(4): 229.
Delis DC, Kaplan E, Kramer JH. 2001. *Delis–Kaplan Executive Function System: Technical Manual*. Psychological Corporation: Pearson - San Antonio, TX.
DeSantis AD, Webb EM, Noar SM. 2008. Illicit use of prescription ADHD medications on a college campus: a multimethodological approach. *J Am Coll Health* **57**(3): 15–324.
Dietz P, Striegel H, Franke AG, *et al.* 2013. Randomized response estimates for the 12-month prevalence of cognitive-enhancing drug use in university students. *Pharmacotherapy* **33**(1): 44–50. DOI:10.1002/phar.1166.
Dodds CM, Bullmore ET, Henson RN, *et al.* 2011. Effects of donepezil on cognitive performance after sleep deprivation. *Hum Psychopharmacol* **26**(8): 578–587. DOI:10.1002/hup.1248.
Downey LA, Kean J, Nemeh F, *et al.* 2013. An acute, double-blind, placebo-controlled crossover study of 320 mg and 640 mg doses of a special extract of *Bacopa monnieri* (CDRI 08) on sustained cognitive performance. *Phytother Res: PTR* **27**(9): 1407–1413. DOI:10.1002/ptr.4864.
Games PA, Klare GR. 1967. *Elementary Statistics; Data Analysis for the Behavioral Sciences*. McGraw-Hill Companies: New York, NY.
Giurgea C. 1972. Pharmacology of integrative activity of the brain. Attempt at nootropic concept in psychopharmacology. *Actual Pharmacol* **25**: 115.
Grön G, Kirstein M, Thielscher A, *et al.* 2005. Cholinergic enhancement of episodic memory in healthy young adults. *Psychopharmacology (Berl)* **182**(1): 170–179. DOI:10.1007/s00213-005-0043-2.
Hirayama S, Terasawa K, Rabeler R, *et al.* 2014. The effect of phosphatidylserine administration on memory and symptoms of attention-deficit hyperactivity disorder: a randomised, double-blind, placebo-controlled clinical trial. *J Hum Nutr Diet* **27**(Suppl 2): 284–291. DOI:10.1111/jhn.12090.
Hirsch C. 2012. Ginkgo biloba extract did not reduce risk for Alzheimer disease in elderly patients with memory complaints. *Lancet Neurol* **11**: 851–859.
Jensen A, Rohwer W. 1966. The Stroop color–word test: a review. *Acta Psychol (Amst)* **25**: 36–93.
Johns MW. 1991. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* **14**(6): 540–545.
Leber P. 1990. Guidelines for the Clinical Evaluation of Antidementia Drugs. Washington, DC, Food and Drug Administration.
Lecrubier Y, Sheehan D, Weiller E, *et al.*. 1997. The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: reliability and validity according to the CIDI. *Eur Psychiatry* **12**(5): 224–231.
Maher B. 2008. Poll results: look who's doping. *Nature* **452**: 674–675.
McCabe SE, Knight JR, Teter CJ, Wechsler H. 2005. Non-medical use of prescription stimulants among US college students: prevalence and correlates from a national survey. *Addiction* **100**: 96–106.
Morasch K, Aaron C, Moon J, Gordon R. 2015. Physiological and neurobehavioral effects of cholinesterase inhibition in healthy adults. *Physiol Behav* **138**: 165–172.
Nie NH, Bent DH, Hull CH. 1975. *SPSS: Statistical Package for the Social Sciences*, Vol **227**. McGraw-Hill: New York.
Nielsen T. 2000. A review of mentation in REM and NREM sleep: "covert" REM sleep as a possible reconciliation of two opposing models. *Behav Brain Sci* **23**(6): 851–866.
Ooyen C. 2012. NBJ's supplement business report, 1–24.
Pace M, Kean J, Neale C, Scholey A, Stough C. 2012. The cognitive-enhancing effects of Bacopa monnieri: a systematic review of randomized, controlled human clinical trials. *J Alternative Compl Med* **18**(7): 647–652.
Reitan R. 1958. Validity of the trail making test as an indicator of organic brain damage. *Probab Math Stat* **8**(7): 271.
Scholey A, Kennedy D, Wesnes K. 2005. The psychopharmacology of herbal extracts: issues and challenges. *Psychopharmacology (Berl)* **179**(3): 705–707. DOI:10.1007/s00213-004-2062-9.
Sheng Y, Li L, Holmgren K, Pero RW. 2001. DNA repair enhancement of aqueous extracts of *Uncaria tomentosa* in a human volunteer study. *Phytomedicine* **8**(4): 275–282. DOI:10.1078/0944-7113-00045.
Shineman DW, Salthouse TA, Launer LJ. 2010. Therapeutics for cognitive aging. *Ann N Y Acad Sci* **1191**(Suppl. 1): E1–E15.
Shook J, Galvagni L, Giordano J. 2014. Cognitive enhancement kept within contexts: neuroethics and informed public policy. *Front Syst Neurosci* 8–228
Skolnick A. 1997. Old Chinese herbal medicine used for fever yields possible new Alzheimer disease therapy. *J Am Med Assoc* **277**(10): 776–776.
Small BJ, Rawson KS, Martin C, *et al.* 2014. Nutraceutical intervention improves older adults' cognitive functioning. *Rejuvenation Res* **17**(1): 27–32. DOI:10.1089/rej.2013.1477.
Smith ME, Farah MJ. 2011. Are prescription stimulants "smart pills"? The epidemiology and cognitive neuroscience of prescription stimulant use by normal healthy individuals. *Psychol Bull* **137**: 717–741.
Solomon PR, Michalczuk DE. 2009. Toward establishing guidelines for evaluating cognitive enhancement with complementary and alternative medicines. *Eval Health Prof* **32**(4): 370–392. DOI:10.1177/0163278709346816.
Solomon P, Adams F, Silver A, Zimmer J, DeVeaux R. 2002. Ginkgo for memory enhancement. *JAMA* **288**(7): 835.
Solomon TM, Solomon PR, Budson AE, Murphy CA. 2014. A proof of concept study for a randomized double-blind, placebo-controlled, parallel group, efficacy study of AlphaBRAIN™ administered orally. Presentation at the 42nd Annual Meeting of the International Neuropsychological Society, Seattle, WA.
Song Z, Han S, Pan X, *et al.* 2015. Pterostilbene mediates neuroprotection against oxidative toxicity via oestrogen receptor α signalling pathways. *J Pharm Pharmacol* . DOI:10.1111/jphp.12360.
Spreen O. 1998. *A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary*. Oxford University Press: New York, NY.
Steenbergen L, Sellaro R, Hommel B, Colzato LS. 2015. Tyrosine promotes cognitive flexibility: evidence from proactive vs. reactive control during task switching performance. *Neuropsychologia* : 6950–55. DOI:10.1016/j.neuropsychologia.2015.01.022.

Strauss E, Sherman EMS, Spreen O. 2006. *A Compendium of Neuropsychological Tests*. Oxford University Press: New York.

Sun QQ, Xu SS, Pan JL, *et al.* 1999. Huperzine-A capsules enhance memory and learning performance in 34 pairs of matched adolescent students. *Acta Pharmacologica Sinica* **20**(7): 601–603.

Tablot M. 2009. Brain Gain. *The New Yorker*. Retrieved from: http://www.iricss.org/fa/NewsRelease/News/Documents/Brain_Gain_The_New_Yorker.pdf [Accessed: 11/9/2014].

Tombaugh T. 2006. A comprehensive review of the Paced Auditory Serial Addition Test (PASAT). *Arch Clin Neuropsychol* **21**(1): 53–76.

Unno K, Tanida N, Ishii N, *et al.* 2013. Anti-stress effect of theanine on students during pharmacy practice: positive correlation among salivary α-amylase activity, trait anxiety and subjective stress. *Pharmacol Biochem Behav* **111**: 128–135. DOI:10.1016/j.pbb.2013.09.004.

Valikovics A. 2007. Investigation of the effect of vinpocetine on cerebral blood flow and cognitive functions. *Ideggyogy Sz* **60**(7–8): 301–310.

Wang S. 2012. 'Medical foods' and supplements for brain health advance—WSJ. [Online]. wsj.com. Available from: http://www.wsj.com/articles/SB10001424052702303919504577524970855874632 [Accessed: 6 March 2015].

Wechsler D. 2009. Wechsler Memory Scale—(WMS-IV).

Wechsler D. 2008. Wechsler adult intelligence scale—Fourth Edition (WAIS–IV). *San Antonio*.

Wechsler D. 2001. *Wechsler Test of Adult Reading: WTAR*. Psychological Corporation: Lutz, FL.

Wodds S, Delis D, Scott J, Kramer J, Holdnack J. 2006. The California Verbal Learning Test—second edition: test–retest reliability, practice effects, and reliable change indices for the standard and alternate forms. *Arch Clin Neuropsychol* **21**(5): 413–420.

Wu C-H, Wang C-C, Tsai M-T, *et al.* 2014. Trend and pattern of herb and supplement use in the United States: results from the 2002, 2007, and 2012 National Health Interview Surveys. *Evid base Compl Alternative Med* **2014**(1): 1–7. DOI:10.1001/jama.279.19.1548.

Xu SS, Cai ZY, Qu ZW, *et al.* 1999. Huperzine-A in capsules and tablets for treating patients with Alzheimer disease. *Acta Pharmacologica Sinica* **20**(6): 486–490.

Xu SS, Gao ZX, Weng Z, *et al.* 1995. Efficacy of tablet huperzine-A on memory, cognition, and behavior in Alzheimer's disease. *Acta Pharmacologica Sinica* **16**(5): 391–395.

Yang Y, Wu Z, Meininger CJ, Wu G. 2015. L-Leucine and NO-mediated cardiovascular function. *Amino Acids* **47**(3): 435–447. DOI:10.1007/s00726-014-1904-y.

Yurko-Mauro K, McCarthy D, Rom D, *et al.* 2010. Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. *Alzheimers Dement* : 1–9. DOI:10.1016/j.jalz.2010.01.013.

Zangara A. 2003. The psychopharmacology of huperzine A: an alkaloid with cognitive enhancing and neuroprotective properties CO in the treatment of Alzheimer's disease. *Pharmacol Biochem Behav* **75**(3): 675–686. DOI:10.1016/S0091-3057(03)00111-4.

Zhang Z, Wang X, Chen Q, *et al.* 2002. Clinical efficacy and safety of huperzine Alpha in treatment of mild to moderate Alzheimer disease, a placebo-controlled, double-blind, randomized trial. *Zhonghua Yi Xue Za Zhi* **82**(14): 941–944.